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Review

Host-selective toxins as agents of cell death in plant–fungus interactions

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SUMMARY

Host-selective toxins are known determinants of compatibility in plant–fungus interactions and provide a powerful model for understanding the specificity of these associations. The identification of genes required for toxin biosynthesis has shown that the genes are unique to the toxin producing species and are clustered in complex loci. These loci may have been acquired by horizontal gene transfer. Many, if not all, host-selective toxins act by disrupting biochemical processes and in several cases the resulting cell death has the characteristics of programmed cell death. This ability to make dead tissue from living has enabled these facultative saprophytic fungi to become plant pathogens.

INTRODUCTION

When a fungal spore encounters a potential host plant, what determines whether the fungus will prove pathogenic or not? For most fungi the answer is largely unknown, certainly in the case where spores encounter a non-host plant, but evidence suggests that the plant's pre-formed defences against pathogen attack, such as cuticle, cell wall and anti-fungal compounds, prevent fungal growth (Heath, 1994, 2000; Osbourn, 1996). This must mean that on host plants the fungus is able to grow despite the host's innate defences, or disable the defences to enable growth.

Fungi can fail to be pathogenic because they induce defences. Induced resistance involves detection of the fungus and the mounting of an increased localized and systemic defence against fungal invasion. This involves the biosynthesis of pathogenesis-related proteins and phytoalexins (Kuc, 1995; van Loon, 1997).

Genetic characterization of specific recognition of rust fungi by flax led to the gene-for-gene principle which proposed that for each gene conferring resistance in the host, there exists a corresponding gene conferring avirulence in the pathogen (Flor, 1955, 1971). Resistance of this type has been characterized, in some cases, by cloning of the resistance gene and the avirulence gene from the species involved (Staskawicz *et al*., 1995). The resistance gene product is thought to be involved in detecting, either directly or indirectly, the gene product of the avirulence gene. Detection is often associated with a hypersensitive response involving localized plant cell death at the point of infection and inhibition of pathogen growth.

The hypersensitive response implicates cell death as a mechanism for resistance, but some necrophytic or facultative saprophytic fungi utilize cell death to their advantage. By secreting toxins which are able to kill cells of susceptible hosts, these fungi are able to infect, colonize and feed off their host and in the meantime complete their own life cycle. In this case, cell death is associated with susceptibility, and resistance is mediated by insensitivity to the effects of the toxin. In most cases, application of purified toxin is sufficient to cause cell death in susceptible plants. These toxins are an absolute requirement for successful infection of susceptible hosts and are therefore compatibility factors (Briggs and Johal, 1994). This implies that by killing the plant cells with a secreted, soluble toxin, the fungi are able to circumvent the innate defences of living plant cells. In all cases examined so far, a single plant gene has conferred a stable form of resistance (or susceptibility) to this type of infection strategy, but several genes are associated with toxin production in the fungus. Thus the gene-for-gene interaction model does not apply for this type of plant–fungus interaction. The toxins, and host sensitivity to them, are a major determinant of the fungus' host range and so they are commonly referred to as host-selective toxins (Pringle and Scheffer, 1964).

The genera *Alternaria* and *Cochliobolus* contain species that produce a variety of host-selective toxins and as a result have different host ranges. These species provide a unique model for understanding host-selectivity and the strategies deployed by pathogens to allow infection. Both genera also contain saprophytic species, or usually isolates of the same species, which have

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Table 1 Fungi producing host-selective toxins: structure, genes known to be required for biosynthesis and genomic organization.

Organism	Toxin	Structure[1]	Known genes for biosynthesis	Locus, identified chromosome
C. carbonum	HC-toxin	Cyclic tetrapeptide	*HTS1*[2], *TOXA*[3], *TOXC*[4], *TOXD*[5], *TOXE*[5], *TOXF*[6], *TOXG*[7]	*TOX2* locus, 3.5 or 2.2 Mbp chromosome[8]
C. heterostrophus	T-toxin	Polyketide	*PKS1*[9], *DEC1*[9]	*TOX1A* and *TOX1B* loci, on translocated chromosomes 12;6 and 6;12[10]
C. victoriae	Victorin	Cyclic pentapeptide	Unknown	*TOX3*[11], localization unknown
A. alternata f.sp. *lycopersici*	AAL-toxin	Aminopentol ester	Unknown	Unknown
A. alternata f.sp. *kikuchiana*	AK-toxin	Epoxy-decatrienoic acid backbone	*AKT1*[12], *AKT2*[12], *AKTR-1*[13], *AKT3-1*[13], *AKTR-2*[13], *AKT3-2*[13]	AKT locus, 4.1 Mbp chromosome[13]
A. alternata f.sp. *fragariae*	AF-toxin	Epoxy-decatrienoic acid backbone	*AKT1*, *AKT2* and *AKT3* homologues[14,12]	Unknown
A. alternata f.sp. *citri tangerine*	ACT-toxin	Epoxy-decatrienoic acid backbone	*AKT1*, *AKT2* and *AKT3* homologues[14,12]	Unknown
A. alternata f.sp. *mali*	AM-toxin	Four member cyclic depsipeptide	*AMT*[15]	*AMT* locus, 0.7 Mbp chromosome[16]

References cited numerically in Table: 1. Walton (1996) 2. Panaccione *et al.* (1992) 3. Pitkin *et al.* (1996) 4. Ahn and Walton (1997) 5. Ahn and Walton (1998) 6. Cheng *et al.* (1999) 7. Cheng and Walton (2000) 8. Ahn and Walton (1996) 9. Yoder (1998) 10. Kodama *et al.* (1999) 11. Walton and Panaccione (1993) 12. Tanaka *et al.* (1999) 13. Tanaka and Tsuge (2000) 14. Masunaka *et al.* (2000) 15. Johnson *et al.* (2000) 16. Akamatsu *et al.* (1999).

no known specific plant host. We are curious to understand how the pathogenic strains are related to the saprophytic strains and what causes their difference in lifestyle. In an analogous fashion, the host range of the toxin producing fungi is limited to specific varieties of the host species. How do the toxins cause cell death and how is it that certain varieties are resistant to the host-selective toxin producing fungi? This relationship between host-selective toxin producing fungi and their hosts provides a unique opportunity to study the origin and evolution of pathogenicity in fungi. Previous reviewers have detailed the structure, biochemistry, and mode of action of these toxins (Knogge, 1996; Nishimura and Kohmoto, 1983; Otani *et al.*, 1995; Pringle and Scheffer, 1964; Walton and Panaccione, 1993; Walton, 1996; Yoder, 1980). In this review we will examine recent advances in the molecular genetics of toxin production and ask whether this helps us to understand how toxin producing strains arose. We will also examine new developments in our understanding of the mode of toxin action and discuss the mechanisms of resistance towards toxins featuring in host-selectivity.

Toxins promoting pathogenicity of *Cochliobolus* and *Alternaria* species

These genera contain several pathogens which show enhanced pathogenicity towards their hosts. The enhanced pathogenicity in these species is linked to toxin production, because nontoxin producing strains can be just as pathogenic as toxin producing strains if they are inoculated on toxin-sensitive host varieties in the presence of the host-selective toxin (Comstock and Scheffer, 1973). Toxins are usually a variety of acyl or ester derivatives of common structures with varying degrees of toxicity. As it is not always clear if a single molecular species is being used and because there is no consensus in the literature on the use of plural vs. singular names, we refer to all toxins in this review in the singular. Whilst toxins are required for pathogenicity, they are not necessarily the sole determinants of pathogenicity. Extracellular proteins, enzymes and non-host selective toxins may all play a role in overall pathogenicity (Annis and Goodwin, 1997; Rotem, 1994; Tonukari *et al.*, 2000; Yoder, 1998).

It is vital for our understanding of these diseases to know what genetic changes have created the enhanced pathogenicity. Recent progress in the isolation of genes involved in toxin biosynthesis has shown that the arrangement of toxin biosynthesis genes follows a similar pattern in this diverse range of fungi (summarized in Table 1), and suggests a common mechanism by which toxin producing strains may have arisen.

Cochliobolus species

The *Cochliobolus* species are common pathogens of monocotyledons, which includes important crop species such as rice, maize and oat (Agrios, 1997). *Cochliobolus* (anamorph *Bipolaris*) species are true fungi of the *Ascomycota*. Most isolates of *Cochliobolus* are non- to only weakly pathogenic, but strains with increased pathogenicity due to the production of host-selective toxins have emerged.

HC-toxin

Leaf spot disease of maize is caused by the pathogen *C. carbonum*. Races 2 and 3 are weakly virulent, producing small chlorotic or necrotic flecks on leaves, but race 1 is extremely pathogenic on

susceptible varieties causing large necrotic lesions on leaves (Pitkin *et al.*, 2000; Welz and Leonard, 1993). The increased pathogenicity of race 1 is due to the production of HC-toxin, a cyclic tetrapeptide containing D-amino acids (Kawai *et al.*, 1983). It was the first host-selective toxin to be identified and consequently provides a model for studies concerning other host-selective toxins.

The genes for HC-toxin biosynthesis have been mapped to a single locus, *TOX2*, which, depending upon the isolate, is located on either a 3.5 Mbp or 2.2 Mbp chromosome (Ahn and Walton, 1996). The locus covers at least 540 kbp and is thought to contain all the genes required for toxin biosynthesis. The central gene for toxin biosynthesis encodes the enzyme HC-toxin synthetase (*HTS1*), a 570 kDa nonribosomal peptide synthase (Panaccione *et al.*, 1992). Other genes, *TOXC*, *TOXD*, *TOXF* and *TOXG*, encode enzymes which may synthesize the substrates for HC-toxin synthetase (Ahn and Walton, 1997; Cheng and Walton, 2000; Cheng *et al.*, 1999). *TOXA* is closely linked to *HTS1* and encodes a putative HC-toxin efflux carrier (Pitkin *et al.*, 1996). All these genes, except *HTS1*, are under the transcriptional influence of *TOXE* (Ahn and Walton, 1998).

The organization of the *TOX2* locus is complex. All the identified genes involved in toxin biosynthesis other than *TOXE* are present in multiple copies. Although the *TOX2* locus was originally thought to be a single Mendelian locus, this is clearly not the case. Crosses between $Tox2^+$ and $Tox2^-$ strains show inheritance of $Tox2^+$ in a Mendelian fashion because there is no homologous locus in naturally isolated $Tox2^-$ strains. Crossover events within the *TOX2* locus are apparently suppressed (Ahn and Walton, 1996), but the actual number of crossover events observed may be reduced by the instability of the locus during meiosis (Pitkin *et al.*, 2000). It remains to be proven if the entire chromosome on which the *TOX2* locus resides is an essential *C. carbonum* chromosome, certainly large parts of it are not required for growth in culture (Ahn and Walton, 1996; Pitkin *et al.*, 2000), or whether it can be thought of as a complete supernumerary chromosome (Covert, 1998). The isolation of markers further away from the *TOX*2 locus on the same chromosome can possibly help to answer this question.

T-toxin

T-cytoplasmic male sterile (T-cms) maize is sensitive to T-toxin, a polyketide synthesized by the fungus *C. heterostrophus*, which is responsible for Southern Corn Leaf Blight disease (Wise *et al.*, 1999). The genes for T-toxin biosynthesis are located at the locus *TOX1* (Leach *et al.*, 1982), which has become separated by a chromosome translocation into two loci, *TOX1A* found on chromosome 12;6 and *TOX1B* on chromosome 6;12 (Kodama *et al.*, 1999; Turgeon *et al.*, 1995). The loci are inseparably linked to a four-armed linkage group which causes the *TOX1* locus to inherit as a single locus (Chang and Bronson, 1996; Tzeng *et al.*, 1992). Karyotype comparisons between race T and non T-toxin producing race O show 1.2 Mbp more DNA in race T which maps to the *TOX1A* and *TOX1B* loci. Two genes have been identified which are necessary for T-toxin biosynthesis, a polyketide synthase (*PKS1*) that is a multifunctional enzyme similar to fatty acid synthases, and *DEC1* which is thought to act as a decarboxylase to remove one carbon as CO_2 from the even numbered carbon chain to produce the odd numbered final T-toxin (Yoder, 1998). Targeted disruption of either *PKS1* or *DEC1* leads to a Tox^- phenotype but the original *pks*1 mutants generated by restriction enzyme-mediated integration (REMI) cannot be complemented by introducing a full-length functional clone of *PKS1* (Kodama *et al.*, 1999). This indicates that additional genes have been mutated in the REMI generated mutant that are required for T-toxin biosynthesis. There have also been reports of other genes affecting T-toxin biosynthesis that are not linked to *TOX1A* or *TOX1B*, but their contribution to toxin biosynthesis remains unclear (Bronson, 1998; Yoder, 1998).

Neither *PKS1* nor *DEC1* can be detected in *C. heterostrophus* races or *Cochliobolus* species which do not produce T-toxin (Wise *et al.*, 1999; Yoder, 1998). However, another fungus, *Mycosphaerella zeae-maydis*, also infects T-cms maize utilizing a polyketide toxin (PM-toxin) that is similar to T-toxin. Recently using REMI mutagenesis, a PKS gene from *M. zeae-maydis* was cloned which, when disrupted, leads to loss of PM-toxin production and virulence (Yoder, 1998; Yun *et al.*, 1998). These two PKSs are 62% homologous at the nucleotide level implying that they are only distantly related.

Victorin

The fungus *Cochliobolus victoriae* causes Victoria blight disease in oats. The fungus secretes a toxin known as victorin that is toxic to susceptible oat leaves at very low concentrations (Wolpert *et al.*, 1995). Victorin is an unusual, halogen containing, cyclic pentapeptide. Toxin synthesis is inherited at a single locus *TOX3* (Walton and Panaccione, 1993), and it would seem reasonable to hypothesize that it is synthesized in a manner analogous to other cyclic peptide toxins, that is, by a non-ribosomal (cyclic) peptide synthetase (Nikolskaya *et al.*, 1995). Enzymes must also be present to synthesize the novel amino acids required by such a peptide synthetase, but unfortunately to date no reports on the identification of any genes necessary for victorin synthesis are known.

Alternaria species

The fungal genus *Alternaria* contains at least 10 host-selective toxin producing plant pathogens which are all pathogens of dicotyledons (Akamatsu *et al.*, 1999; Otani *et al.*, 1995). The largest group of pathogens are proposed to be all pathotypic variants of the species *Alternaria alternata*, a very common, imperfect fungus (Nishimura and Kohmoto, 1983). Molecular phylogenetics by analysis of DNA–DNA re-association kinetics, rDNA regions and internal

transcribed spacer regions from different *Alternaria alternata* pathotypes supports this proposal (Kusaba and Tsuge, 1994, 1995). Consequently, we use the system of formae speciales suggested by Rotem (Rotem, 1994), which defines the *A. alternata* subspecies according to host-specificity.

AAL-toxin

The fungus *Alternaria alternata* f.sp. *lycopersici* causes *Alternaria* stem canker of susceptible tomatoes (Grogan *et al*., 1975). The fungus produces an aminopentol ester toxin which is very similar in structure to fumonisin, a toxin first identified in *Fusarium moniliforme* (Gilchrist *et al*., 1994). The consumption of food contaminated by fumonisin is implicated in the development of several human and animal diseases (Nelson *et al*., 1993).

Identification of the genes involved in AAL-toxin biosynthesis is not possible by traditional genetic methods because *A. alternata* has no known sexual cycle. By using REMI however, it has been possible to create AAL-toxin deficient mutants (Akamatsu *et al*., 1997; Kodama *et al*., 1998). These mutants are not pathogenic, confirming the requirement of the toxin for pathogenesis. The number of mutants recovered, 1 per 100 screened, suggests that there may be many genes involved, or that there is instability in the locus during the REMI transformation procedure. Isolation of the sequences adjacent to the insert has not been reported in *A. alternata* f.sp. *lycopersici*, but this technique has been successful for other *A. alternata* pathotypes.

AM-toxin

Alternaria blotch is a worldwide disease of susceptible apple caused by *A. alternata* f.sp. *mali* which is known to produce AM-toxin, a four-member cyclic depsipeptide. Cloning of the genes involved in toxin biosynthesis is underway with the isolation of a gene for AM-toxin synthetase (*AMT*), a potential cyclic peptide synthetase (Johnson *et al*., 2000). One of the most homologous genes of AM-toxin synthetase is HC-toxin synthetase from *C. carbonum*. Similarly, as for HC-toxin synthetase, there is also evidence for more than one copy of *AMT* in the *A. alternata* f.sp. *mali* genome located together on one chromosome (Akamatsu *et al*., 1999; Johnson *et al*., 2000). Such an arrangement of genes is highly similar to the characterized *TOX2* locus for HC-toxin biosynthesis. It will be interesting to see whether the small chromosome of *A. alternata* f.sp. *mali* contains a locus with all the genes necessary for AM-toxin biosynthesis and is also unique to the apple pathotype.

AK-toxin

Alternaria alternata f.sp. *kikuchiana* causes black spot on susceptible varieties of Japanese pear. It produces AK-toxin which is an epoxy-decatrienoic ester. AK-toxin deficient insertion mutants created by REMI allowed the identification and cloning of two genes, *AKT1* and *AKT2* (Tanaka *et al*., 1999). *AKT1* shows homology to a diverse group of enzymes, all of which are ATP-dependent, carboxyl-activating enzymes many of which are involved in lipid metabolism and the attachment of acyl chains to CoA. This suggests a role for *AKT1* in the biosynthesis of the decatrienoic backbone of AK-toxin. *AKT2* was not identified as having homology to any known proteins, but a current PSI-BLAST search reveals homology to a new group of enzymes with an alpha/beta hydrolase fold. The representative member of this group is an epoxide hydroxylase but it also contains lipases, acetyltransferases and oxidoreductases. Involvement of any such enzymes in AK-toxin biosynthesis awaits further investigation. Examination of a 7 kbp region downstream of *AKT2* identified two further open reading frames, *AKTR-1* and *AKT3-1* (Tanaka and Tsuge, 2000). *AKTR-1* contains domains typical of fungal regulatory factors and hence is postulated to be involved in regulation of *AKT* gene(s) expression, perhaps in a manner analogous to *TOXE* from *C. carbonum*. *AKT3-1* encodes a protein with similarity to hydratase/isomerase enzymes such as enoyl-CoA hydratase. Targeted disruption experiments produced inserts only in highly similar genes subsequently called *AKTR-2* and *AKT3-2*. These disrupted mutants were Tox^{-}, indicating that they are essential for toxin biosynthesis. All of these genes have multiple homologues in *A. alternata* f.sp. *kikuchiana*, but nonetheless, all the genes map to a single 4.1 Mbp chromosome (Tanaka and Tsuge, 2000).

Other *Alternaria alternata* pathotype toxins

A. alternata f.sp. *fragariae*, which infects strawberry, and *A. alternata* f.sp. *citri*, pathotype tangerine, produce toxins with epoxy-decatrienoic ester backbones, designated AF- and ACT-toxin, respectively (Otani *et al*., 1995). This structure is also present in AK-toxin, indeed certain forms of AF- and ACT-toxin are also toxic to AK-toxin susceptible cultivars of Japanese pear. Cross pathotype hybridization studies and PCR cloning have revealed that the strawberry and tangerine pathotypes contain homologues of the genes implicated in AK-toxin biosynthesis (Masunaka *et al*., 2000; Tanaka *et al*., 1999). The arrangement of the loci and structure of other genes involved in toxin biosynthesis in these fungi awaits further research.

COMPATIBLE INTERACTIONS BETWEEN HOST-SELECTIVE TOXIN PRODUCING FUNGI AND HOSTS

The early events in infection by *Cochliobolus* or *Alternaria* species are very similar; both require free moisture for germination and high humidity for infection to spread (Rotem, 1994). Once a germ tube has emerged, the plant is penetrated through wounds,

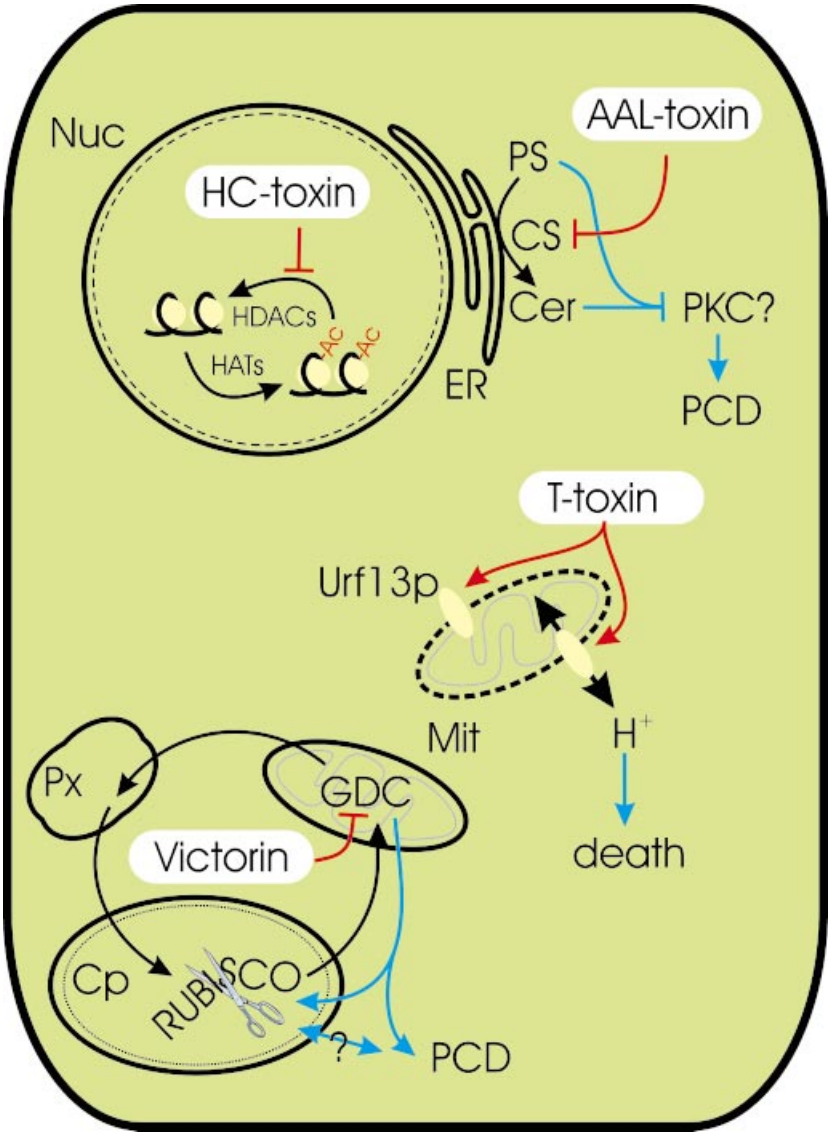

Fig. 1 Known cellular targets and possible mechanisms for host-selective toxins. **HC-toxin** acts in the nucleus (Nuc) where it is known to inhibit histone deacetylases (HDACs) creating a hyperacetylation of histones through histone acyltransferases (HATs). This is thought to lead to changes in gene expression which prevent the synthesis of antifungal compounds. **AAL-toxin** inhibits ceramide synthase (CS), an enzyme of the endoplasmic reticulum (ER), which catalyses the synthesis of ceramide (Cer) from phytosphingosine (PS) and palmitoyl-CoA. Both ceramide and phytosphingosine are bioactive compounds capable of altering protein kinase C (PKC) activity in animals which can lead to programmed cell death (PCD). It is not known if protein kinases are inhibited in a similar way in the plants. **T-toxin** is a ligand for the novel protein Urf13p, present in T-cms mitochondrial membrane (Mit). Binding of T-toxin is thought to cause pore formation or opening leading to loss of ion gradients (e.g. the H^+ gradient) across the mitochondrial membrane and cell death. **Victorin** is a potent inhibitor of glycine decarboxylase (GDC) which plays an essential role in the photorespiratory cycle in which products of the oxygenase reaction of RUBISCO are exchanged between the chloroplast (Cp), mitochondrion (Mit) and peroxisome (Px). Victorin treatment leads to cleavage of RUBISCO and PCD. It is not known how RUBISCO cleavage relates to the mechanism of PCD.

stomata, areas of necrotic cells, or directly through the epidermis by formation of an appressorium (Comstock and Scheffer, 1973; Rotem, 1994). These processes are the same in both toxin and non-toxin producing varieties of the fungi, but in non-toxin producing fungi the disease fails to develop beyond the first infection court. As pathogenicity proceeds only in the presence of toxin, the toxin must allow the fungus to overcome or suppress this block in infection.

The simplest way to evade a defence response is to kill plant tissue directly and most of the toxins discussed here do just that (Fig. 1). The exception is HC-toxin, which is unique in that it is not by itself capable of killing cells. Instead, the toxin is able to prevent a response, such as secretion of inhibitory compounds (Cantone and Dunkle, 1991), which would otherwise inhibit further growth (Cantone and Dunkle, 1990; Comstock and Scheffer, 1973). Response suppression is thought to be through the toxins inhibitory effect on histone deacetylases. Changes in histone acetylation are known to alter gene regulation, and this is thought to prevent the plant's response (Ciuffetti *et al.*, 1995). HC-toxin biosynthesis is regulated to coincide with appressorium formation, ensuring that the plant is unable to mount a response from the first moments of infection (Weiergang *et al.*, 1996).

All the other toxins mentioned in this review directly cause cell death. Application of the toxins to sensitive tissues, often in submicromolar concentrations, causes cell death with the characteristic symptoms of the disease. For the majority of toxins the mechanism of cell death has not been molecularly characterized, but for two toxins a common mechanism has emerged,

programmed cell death. Programmed cell death, known as apoptosis in animals, is recognized as an important factor for many pathogen caused animal diseases (Finlay and Cossart, 1997), but its role in plant pathology has only recently been described (Gilchrist, 1998; Greenberg, 1997).

PROGRAMMED CELL DEATH IN RESPONSE TO HOST-SELECTIVE TOXINS

Programmed cell death (PCD) occurs when a cell makes an active contribution towards its own death, whether this is rapid (e.g. hypersensitive response) or slow (e.g. senescence). PCD has various hallmarks which are seen in both animals and plants (Danon *et al.*, 2000; Pennell and Lamb, 1997). The hallmarks are components of a signalling pathway, including reactive oxygen species and cysteine proteases (caspases), that results in internucleosomal DNA cleavage and fragmentation of the nucleus. Inhibitors of cellular processes, such as protein synthesis or energy production, will prevent or stop PCD from taking place. Treatment of susceptible tissues with victorin or AAL-toxin induces molecular and physiological changes associated with PCD. Protein synthesis inhibitors also inhibit cell death when susceptible plants are treated with AK- and AM-toxin, implicating PCD in their mode of action (Otani *et al.*, 1995; Walton, 1996). PCD may be a common feature for many host-selective toxins that are capable of killing susceptible tissues.

AAL-toxin induced PCD

AAL-toxin and its relative fumonisin are structurally related to sphinganine and are inhibitors of sphinganine-N-acyltransferase (ceramide synthase) (Gilchrist *et al.*, 1994). As a result of their structural and functional similarity, AAL-toxin and fumonisin are collectively referred to as sphinganine-analogue mycotoxins or SAMs. The inhibition of ceramide biosynthesis by SAMs has made these toxins a useful tool for studying the role of ceramides and ceramide containing lipids in animal biology (Merrill *et al.*, 1996).

Sphingosine, sphingosine-1-phosphate, ceramide and ceramide containing lipids are bioactive compounds involved in a diverse array of intracellular signalling pathways in animals (Gilchrist, 1997; Hannun and Luberto, 2000; Merrill *et al.*, 1996; Okazaki *et al.*, 1998). When SAMs are applied to cultures of rat hepatocytes, sphingolipid biosynthesis is disrupted and an accumulation of sphinganine is seen followed by apoptosis (van der Westhuizen *et al.*, 1998; Wang *et al.*, 1991). Monkey kidney cells were shown to undergo apoptosis with characteristic DNA strand breaks and nuclear fragmentation when treated with SAMs (Wang *et al.*, 1996a). Similarly, susceptible tomato tissue accumulates increased levels of sphinganine and phytosphingosine when challenged with SAMs (Abbas *et al.*, 1994). Programmed cell death associated features including DNA laddering and fragmentation of the nucleus were shown in protoplasts and leaves of susceptible tomato treated with SAMs (Wang *et al.*, 1996b). SAMs also induced an increase in free sphinganine and phytosphingosine in nonsusceptible tomato leaves, but the increase in sphinganine was five to six-fold less (Abbas *et al.*, 1994). This implies that ceramide synthase is inhibited to a greater extent in AAL-toxin sensitive plants than in nonsensitive plants, although reported *in vitro* measurements of ceramide synthase activity have suggested otherwise (Gilchrist *et al.*, 1994; Gilchrist, 1997). Significantly, AAL-toxin induced cell death can be avoided in sensitive tomato leaflets by feeding ceramide, indicating that a ceramide imbalance is critical in causing cell death (Brandwagt *et al.*, 2000). It is currently not known how SAMs activate programmed cell death in animals or plants but changes in the intracellular concentration of ceramide and sphingoid bases are clearly involved.

Victorin induced PCD

A possible target for victorin induced cell death was identified by isolating a 100 kDa victorin binding protein (Wolpert *et al.*, 1994). The gene encodes a P subunit of the glycine decarboxylase complex (GDC). Glycine decarboxylase is essential for the photorespiratory cycle. Victorin is reported to inhibit GDC at picomolar concentrations when applied to leaf slices and at micromolar concentrations *in vitro* (Navarre and Wolpert, 1995). Although the exact mechanism by which programmed cell death is induced is far from understood, it appears that by inhibiting photorespiration the leaves are rapidly induced to undergo senescence, a form of programmed cell death (Navarre and Wolpert, 1999b). There is a rapid response to victorin which is dose, calcium and light dependent and is characterized by cleavage of RUBISCO, lipid oxidation, DNA laddering characteristic of programmed cell death and production of ethylene. Cleavage of RUBISCO during victorin treatment is dependent upon light and respiration and is inhibited by: (i) increased concentrations of CO_2, which inhibits photorespiration; (ii) La^{2+}, which blocks calcium channels; (iii) ethylene, a plant hormone involved in senescence; (iv) Zn^{2+}, which is known to inhibit PCD activated nucleases; and (v) E64, a cysteine protease inhibitor (Navarre and Wolpert, 1999a,b). These requirements all point to a specific signal transduction pathway that is induced by inhibition of the photorespiration pathway. Likely candidates for initiation of a signal cascade leading to PCD in this case would be the production of reactive oxygen species, which have been strongly implicated in programmed cell death (Lamb and Dixon, 1997), or mitochondrial dysfunction, which is also important in PCD in animals (Desagher and Martinou, 2000). The contribution of either of these potential pathways remains to be assessed either by staining for reactive oxygen species or looking for key changes in the mitochondria, such as permeability transition or cytochrome C release, both associated with PCD in animals and plants.

RESISTANCE AND SUSCEPTIBILITY ALLELES—STRUCTURE AND FUNCTION

Insensitivity to host-selective toxins can be either dominant (HC-toxin), co-dominant (AAL-toxin), recessive (victorin, all other *Alternaria* host-selective toxins) or cytoplasmatic (T-toxin). These different types of resistance alleles demonstrate the variety of mechanisms for insensitivity to toxins.

The gene *Hm1* encodes an enzyme capable of reducing HC-toxin rendering the toxin inactive. *Hm1* is present in all monocotyledons examined, but not in dicotyledons. The role of this enzyme, other than conferring resistance to HC-toxin, is not known. Susceptibility of maize to *C. carbonum* arose through the disruption of both *Hm* alleles, *hm1* was created through a transposon insertion and *hm2 through* a deletion (Multani *et al.*, 1998).

Resistance to AAL-toxin is carried at the *Asc* locus and is co-dominant, meaning an intermediate response is seen in heterozygous plants (van der Biezen *et al.*, 1994). Plants that are susceptible to the fungus are genotype *asc*/*asc* and excised leaves die in a bioassay at concentrations as low as 20 nM AAL-toxin (Witsenboer *et al.*, 1988). Leaves from heterozygous *Asc*/*asc* plants are 10 times less sensitive and require at least 200 nM toxin to cause cell death. These plants are resistant to the fungus, indicating that the fungus cannot secrete toxin *in planta* to create intracellular concentrations this high. In *Asc*/*Asc* leaves, toxin induced cell death can be seen at a concentration of about 2 μM. The gene for *Asc* has recently been cloned (Brandwagt *et al.*, 2000) and our current studies indicate that in yeast it can substitute for the deletion of the genes *LAG*1 and *LAC*1 (Barz and Walter, 1999; S.D. Spassieva and J.E. Markham, unpublished data). These yeast genes have been shown to play a role in GPI-anchored protein transport (Barz and Walter, 1999) and current work indicates that this is because *lag*1/*lac*1 mutants do not show normal ceramide synthase activity (Guillas *et al.*, 2001). By transforming *asc*/*asc* tomato plants with the *Asc* gene, plants become resistant to *A. alternata* f.sp. *lycopersici* and AAL-toxin (J.E. Markham and J. Hille, unpublished data). If the *Asc* gene product is affecting ceramide synthase activity then presumably *asc*/*asc* plants have less ceramide synthase activity than *Asc*/*Asc* or *Asc*/*asc* plants and therefore in the presence of low levels of AAL-toxin are unable to synthesize enough ceramides for cell survival. This also explains the correlation between the copy number of the *Asc* allele and the concentration of applied AAL-toxin at which cell death occurs. The *asc* allele contains several deletions relative to the *Asc* allele, one of which indicates that a non-functional protein would result from translation of the *asc* mRNA (Brandwagt *et al.*, 2000). The role of the *Asc* gene product in normal growth still remains to be determined as *asc*/*asc* plants have no visible phenotype. Physiological studies of *asc*/*asc* plants have demonstrated that they are perturbed in sucrose transport although the origin of this perturbation and how it is related to ceramide biosynthesis remains to be explained (Moussatos *et al.*, 1993).

Sensitivity to victorin, and hence susceptibility to *C. victoriae* is conferred by a dominant gene, *Vb*. This gene is believed to be identical to *Pc*-2 which confers resistance in oat to crown rust disease caused by *Puccinia coronata*. Thus one and the same gene may confer resistance to *P. coronata* but susceptibility to *C. victoriae*. Currently there is no information on the identity of the *Vb*/*Pc*-2 locus so it is not known if the 100 kDa victorin binding protein, identified as a P subunit of GDC, represents the product of the *Vb* allele. Detection of victorin binding proteins in extracts from oat tissues depends on the method used. If a labelled toxin is used, victorin binds to the GDC P subunit only in susceptible cultivars (Wolpert *et al.*, 1994), but if an antibody against victorin is used then binding is detected in both susceptible and resistant cultivars (Akimitsu *et al.*, 1992). Resolving this difference awaits mapping of the GDC P subunit and cloning of the *Vb* allele.

Resistance to T-toxin is dependent upon the absence of Urf13 protein in the mitochondrial membrane. *URF13* is a chimeric gene created by a rearrangement in the mitochondrial genome of T-cms maize (Dewey *et al.*, 1986). The gene is derived from a fragment of the mitochondrial 26S ribosomal gene which has undergone many point mutations, deletions, insertions and rearrangements to produce an open reading frame which encodes a novel 13 kDa protein. This protein has three transmembrane helices and forms a tetrameric ligand gated ion channel in the inner mitochondrial membrane, the activating ligand in this case is T-toxin. The protein is also able to confer T-toxin sensitivity to *E. coli* and *S. cerevisiae* demonstrating that it is able to form channels in a variety of biological systems. Normally male fertile maize is resistant to *C.heterostrophus* because *URF13* is absent from the mitochondrion. Resistance in T-cms maize can also be created by the nuclear suppressor genes, *Rf*-1 and *Rf*-2, which are able to suppress the effects of *URF13*. *Rf*-1 makes this possible by altering the expression of *URF13* through transcriptional processing causing a reduction in Urf13 protein production. *Rf*-2 encodes an aldehyde dehydrogenase that may enable alternative metabolic pathways to occur in T-cms mitochondria.

INTERACTIONS WITH NON-HOST PLANTS

The name 'host-selective toxins' implies that these toxins are the sole, or major, determinants of specificity in these plant–fungus interactions. This is demonstrated by the pathogenicity of *C. victoriae*, normally pathogenic towards oat, on HC-toxin sensitive maize in the presence of HC-toxin (Comstock and Scheffer, 1973). It would be interesting to see if introduction of the *Vb* gene into maize confers susceptibility to *C. victoriae*. Expression of the Urf13 protein in *E. coli*, and when targeted to the mitochondria in *S. cerevisiae*, confers sensitivity to T-toxin, suggesting that expression of Urf13 protein in the mitochondria of other

species would also make them sensitive to T-toxin. Does sensitivity to a host-selective toxin always correlate with susceptibility to the toxin producing pathogen?

As a general inhibitor of ceramide synthase, AAL-toxin and its close relative fumonisin are toxic to a variety of plants and animal cells. A survey of a representative sample of *Solanaceae* species shows that about 12% of species are sensitive at 0.2 μM AAL-toxin and 2.5% are as sensitive as *asc*/*asc* tomato (Mesbah *et al*., 2000). A study of the *Nicotiana* genus revealed five species which were sensitive to 0.2 μM AAL-toxin and one species which was colonized by *A. alternata* f.sp. *lycopersici*, indicating that other plants can serve as hosts for this fungus (Brandwagt *et al*., 2001). The other four AAL-toxin sensitive species were not hosts for *A. alternata* f.sp. *lycopersici* indicating that other factors are also involved in host-selection. The structure of the *Asc* homologues in these sensitive species is not known, but it would be interesting to see if disruptions of homologues in other species, or homologues of *Asc* in tomato, create an AAL-toxin sensitive phenotype. Preliminary studies in our lab suggest that AAL-toxin sensitive plants can indeed be created in this way (J.E. Markham and J. Hille, unpublished data).

CONCLUSIONS

Comparison of the genetic organization of genes for host-selective toxin biosynthesis within the *Cochliobolus* and *Alternaria* species shows that they are physically clustered together in the genome and are usually unique to the toxin producing pathotype. Although clustering may be the result of normal, although unclear, sexual or parasexual processes, it has been suggested that the genes for toxin biosynthesis are acquired *en bloc* by horizontal gene transfer (Ahn and Walton, 1996; Rosewich and Kistler, 2000; Tanaka *et al*., 1999; Walton, 2000; Yang *et al*., 1996). This is an accepted mechanism for pathogen evolution amongst the prokaryotes, but remains to be demonstrated in eukaryotes. Nonetheless, it still does not help us understand if the fungi evolve to become pathogenic to plants after the susceptibility genes have evolved (or been bred in), or if the fungi already have the ability to synthesize these compounds and merely exploit the toxin sensitivity of such plants. Pathogenic fungi may be present before known suitable hosts (Wise *et al*., 1999) and may persist for many years once the hosts are removed (Gilchrist *et al*., 1994; Grogan *et al*., 1975). In such instances the selective pressure for toxin production could be antifungal activity towards competitors. The mycotoxins australifungin and fumonisin are both thought to target the same enzyme as the host-selective toxin AAL-toxin, and both have antifungal activity (Keyser *et al*., 1999; Mandala *et al*., 1995). Alternatively, there could be other hosts for these fungi of which we are currently unaware.

All of the host-selective toxins whose protein targets have been characterized, act by altering the properties of their targets, leading to cellular dysfunction, and not by a receptor-mediated, elicitor-induced, signal transduction pathway. By this mechanism, they are able to cause cell death and/or susceptibility to infection in the target tissues. They highlight the importance of previously poorly understood processes in cell homeostasis and provide an invaluable tool for plant biologists. Recent results have pointed towards a common result of host-selective toxin disruption of cell homeostasis, namely, programmed cell death. It has not been conclusively demonstrated that programmed cell death plays a role in cell death caused by other toxins, but it appears feasible. Does the fact that these toxins cause programmed cell death tell us anything about the mechanism of pathogenicity? Perhaps only by inducing programmed cell death, a normal cellular process, is the fungus able to evade the response from the plant that a necrotic attack may bring.

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